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Review

Wnt signal transduction and the formation of the myocardium

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Abstract

Soon after fertilization, vertebrate embryos grow very rapidly. Thus, early in gestation, a sizeable yet underdeveloped organism requires circulating blood. This need dictates the early appearance of a contractile heart, which is the first functional organ in both the avian and mammalian embryo. The heart arises from paired mesodermal regions within the anterior half of the embryo. As development proceeds, these bilateral precardiac fields merge at the midline to give rise to the primary heart tube. How specific areas of nondifferentiated mesoderm organize into myocardial tissue has been a question that has long intrigued developmental biologists. In recent years, the regulation of Wnt signal transduction has been implicated as an important event that initiates cardiac development. While initial reports in *Drosophila* and the bird had implicated Wnt proteins as promoters of cardiac tissue formation, subsequent findings that the WNT inhibitors Dkk1 and crescent possess cardiac-inducing activities led to the contrary hypothesis that WNTs actively inhibit cardiogenesis. This seeming contradiction has been resolved, in part, by more recent information indicating that Wnts stimulate multiple signal transduction pathways. In this review, we will examine what is presently known about the importance of regulated Wnt activity for the formation of the heart and the development of the myocardium and discuss this information in context of the emerging complexity of Wnt signal transduction.

Keywords: Wnt; Dickkopf; Frizzled-related proteins; Cardiac development; Myocardium; Heart fields

Introduction

A functional heart forms very early during mammalian and avian embryogenesis as the survival of a rapidly growing organism requires circulating blood. In contrast, aquatic amphibian and fish embryos undergo substantial development prior to establishing a functional cardiovascular system. Yet, these stage variances in cardiac function reflect not a delay, but a prolongation of heart development in aquatic embryos. In all vertebrates, heart formation begins during the onset of gastrulation. Cells fated to become heart localize to paired mesodermal regions within the anterior half of the embryo. As development proceeds, these bilateral precardiac fields merge at the midline to give rise to the primary heart tube.

Why and how specific areas of nondifferentiated mesoderm organize into cardiac tissue are questions that have intrigued developmental biologists for generations. A vocation of many cardiac biologists has been to identify the molecular cues that provoke the formation of the vertebrate heart. The three signals that have received the most attention as primary stimuli of cardiogenesis are regulators of FGF, BMP, and Wnt signal transduction. This review will focus on the importance of Wnts and the regulation of their activity for initiating vertebrate cardiogenesis. We first begin with a description of Wnt proteins and a brief synopsis of Wnt signal transduction. Next, we describe the known patterns of expression of Wnts and Wnt inhibitors during the stages when the precardiac mesoderm is specified and within the developing primary heart, which have helped identify candidate regulators of cardiogenesis. An overview will be provided of the functional studies that established the importance of Wnt signal transduction for cardiac development, including the hypotheses that have been proposed to explain the functional relevance of specific Wnts and Wnt inhibitors for formation of the heart. Finally, we will bring into the discussion experimental data that may not fit comfortably

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with current theory on Wnt signaling and heart formation and subsequently present an alternative hypothesis that reflects more recent data on the role of WNT signaling in regulating embryogenesis.

Wnt signal transduction

This overview of Wnt signal transduction will start with a disclaimer. The description of Wnt signaling pathways provided below is neither meant to be a comprehensive narrative nor an account of the latest, cutting-edge findings on Wnt signal transduction. The purpose here is to supply sufficient detail for the reader to understand the signal transduction issues that are relevant to a discussion of cardiac developmental biology. Additional information on the complexities of Wnt regulatory activity can be obtained from many excellent reviews and essays (Bejsovec, 2005; Kühl, 2004; Logan and Nusse, 2004; Tolwinski and Wieschaus, 2004). Another useful resource is 'The Wnt Homepage' at http://www.stanford.edu/~rnusse/wntwindow.html.

Wnts are a family of secreted signaling proteins, which have a major influence on embryonic development, tumor progression, and stem cell differentiation (Logan and Nusse, 2004; Moon et al., 2004; Reya and Clevers, 2005). In the mammalian genome, 19 distinct Wnt genes have been identified. At first, Wnt functional activity was thought to be due solely to an individual signal transduction pathway (Fig. 1), which is now referred to as the canonical Wnt pathway (Logan and Nusse, 2004; Wang and Wynshaw-Boris, 2004). This pathway is triggered by Wnt binding to its cell membrane receptor, which comprises the 10 known members of the frizzled family of transmembrane proteins (Huang and Klein, 2004; Logan and Nusse, 2004). The signal from this ligand/receptor interaction is transduced by the cytoplasmic protein Dishevelled (Dsh), which results in the inactivation of glycogen synthase kinase 3 (GSK3). Inhibition of this kinase prevents the phosphorylation of β-catenin. In turn, β-catenin is then able to bind to LEF/TCF transcription factors, forming a complex that translocates to the nucleus and exerts transcriptional enhancer activity (Logan and Nusse, 2004; Wang and Wynshaw-Boris, 2004). Other key molecules in this pathway include: the low-density lipoprotein receptor-related protein (LRP) isoforms LRP5 and LRP6, which function as co-receptors for Wnt binding to the cell membrane (He et al., 2004); and Axin, which enhances GSK3 phosphorylation of β-catenin by forming a cytoplasmic complex with these other proteins (Luo and Lin, 2004). Wnt binding to LRP5/6 influences Axin distribution, thereby assisting Wnt inhibition of GSK3 activity (Mao et al., 2001b).

During the past few years, the conception of Wnt signal transduction has evolved considerably. It is now accepted that alternative noncanonical Wnt signaling pathway(s) exist (Fig. 2), which involve G-proteins, the transmembrane protein strabismus, phospholipase C (PLC), protein kinase C (PKC), Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), c-jun kinase (JNK), Rho family GTPases, and intracellular Ca^{2+} release (Keller, 2002; Kühl, 2004; Park and Moon, 2002; Sheldahl et al., 1999, 2003; Veeman et al., 2003). Because the identification of noncanonical Wnt signaling is relatively recent, many molecular details have not yet been fully elucidated. Often, noncanonical Wnt signaling is described as representing two distinct pathways, which are the Wnt/Ca^{2+} and Wnt/planar cell polarity (PCP) pathways (Fanto and McNeill, 2004; Keller, 2002; Kühl, 2004). However, plausible models have also been proposed that incorporate these disparate components into a single pathway or Wnt regulatory network (Kühl et al., 2001; Veeman et al., 2003).

An additional level of complexity to Wnt signaling is that there is a divergence of functional activities among members of

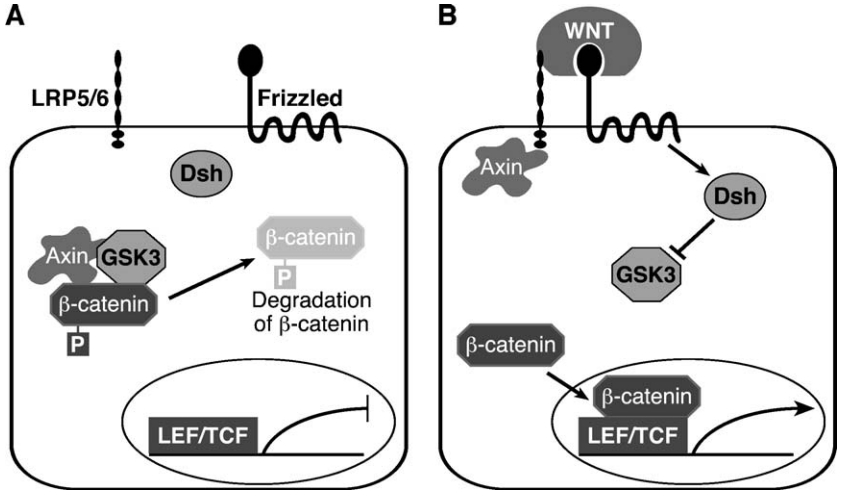

Fig. 1. Schematic diagrams depicting the canonical Wnt signal transduction pathway. (A) In the absence of Wnt protein, β-catenin is sequestered within a multiprotein protein complex that includes glycogen synthase kinase 3 (GSK3) and Axin. GSK3 phosphorylation of β-catenin targets the latter protein for degradation by ubiquination. (B) Wnt binding to frizzled and LRP5/6 transmembrane proteins stimulates the cytoplasmic protein Dishevelled (Dsh), which results in the inactivation of GSK3. This in turn prevents the phosphorylation of β-catenin, which allows its association with LEF/TCF transcription factors, forming a complex that translocates to the nucleus and exerts transcriptional enhancer activity. More detailed information on canonical WNT signal transduction can be found in 'The Wnt Homepage' at www.stanford.edu/~rnusse/wntwindow.html.

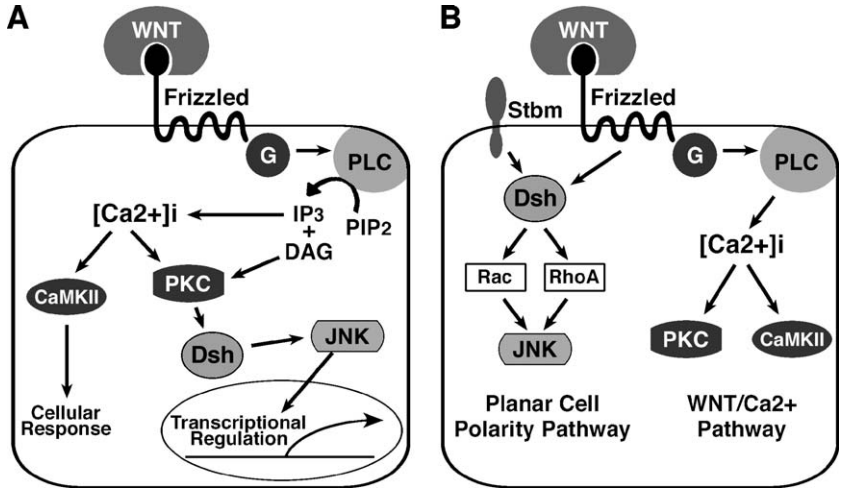

Fig. 2. Alternative pathway(s) of WNT signal transduction. (A) Depiction of a single noncanonical Wnt pathway involving G-proteins, phospholipase C (PLC), protein kinase C (PKC), c-jun kinase (JNK), Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), and Dsh. Key features include the hydrolysis of phosphatidylinositol bisphosphate (PIP2) by PLC to produce diacylglycerol (DAG) and inositol triphosphate (IP3), which in turn promotes intracellular Ca^{2+} release. Furthermore, the activation of PKC may regulate Dsh protein, which subsequently stimulates JNK activity. (B) Other evidence suggests that noncanonical Wnt signaling may be separated into two distinct pathways, which are the planar cell polarity (PCP) and Wnt/Ca^{2+} pathways. Molecules that may distinguish the PCP pathway are: the transmembrane protein strabismus (stbm), which is a putative regulator of Dsh; and the Rho family GTPases, Rac, and RhoA, which stimulate JNK activity. Note that Dsh in involved in both canonical and noncanonical Wnt pathways, which may be due to altering its activities by differential phosphorylation. Please refer to the text for references that provide a more in depth description of these signal transduction events.

this protein family. This diversity has led to the classification of Wnts into two separate groups (Du et al., 1995; Moon et al., 1997). The Wnt1 group, which includes Wnt1, Wnt3a, and Wnt8, appears to signal exclusively via the canonical Wnt/β-catenin pathway (Logan and Nusse, 2004). In contrast, the Wnt5a group, which comprises Wnt4, Wnt5a, and Wnt11, possesses more complex signaling properties. These molecules do not normally trigger the canonical Wnt pathway but instead initiate noncanonical Wnt signaling (Du et al., 1995; Maurus et al., 2005; Pandur et al., 2002; Slusarski et al., 1997; Tada and Smith, 2000). Moreover, signals provided by the Wnt5a group (including Wnt11) have been shown to suppress β-catenin-mediated signaling, due to the regulatory inhibition by the noncanonical Wnt pathway (Maye et al., 2004; Topol et al., 2003; Torres et al., 1996; Weidinger and Moon, 2003; Westfall et al., 2003). Thus, this subgroup of Wnt proteins can function as dominant negative inhibitors of Wnt1 class proteins (Fig. 3). Yet, as a further complication, changes in Frizzled isoform expression can alter the signaling properties of the Wnt5a group from being inhibitors into activators of the β-catenin pathway (He et al., 1997; Lyons et al., 2004).

Several types of canonical Wnt inhibitors have been described, including two distinct families of soluble factors that are secreted into the extracellular environment (Fig. 3). Secreted frizzled-related proteins (sFRP) are soluble proteins that are related to, but genetically distinct from, the frizzled cell membrane receptor proteins (Kawano and Kypta, 2003; Rattner et al., 1997). Since the Wnt binding domain is conserved among sFRP and frizzled proteins, the former exert their activity by competitively inhibiting the binding of Wnts to their cell membrane receptors. In addition, sFRPs can attach to frizzleds as heteromers, which may also play a role in their inhibitory activity (Bafico et al., 1999). To date, six distinct sFRP genes have been discovered in the mouse genome, which include sFRP3 (also known as Frzb1) and the molecule referred to as crescent (Marvin et al., 2001; Pera and De Robertis, 2000; Shibata et al., 2000). Dickkopf (Dkk) proteins comprise a group of four related molecules, which have been primarily characterized by their inhibition of canonical Wnt signaling (Glinka et al., 1998; Kawano and Kypta, 2003). These molecules share with the Wnts the ability to bind the cell membrane receptors LRP5 and LRP6 (Mao et al., 2001a; Semenov et al., 2001). Thus, competitive binding to the extracellular domain of LRP5 and LRP6 may serve as their mechanism of canonical Wnt inhibition, although Dkk signaling via LRP5 and LRP6 may also play a role in their regulation of Wnt activity (Bafico et al., 2001). In addition, Dkk proteins interact with a second group of receptors that regulate their effects on Wnt activity, which are the Kremen transmembrane proteins (Davidson et al., 2002; Mao et al., 2002).

Expression of Wnts and Wnt inhibitors at the onset of heart formation

In vertebrate species, WNT gene expression is displayed from the beginnings of embryogenesis (Table 1). For example, in the prestreak chick embryo, both Wnt5a and Wnt8 are detected in the area opaca, while Wnt11 is detected throughout the area pellucida (Skromne and Stern, 2001). Chick Wnt1 and Wnt3A are also detected in prestreak embryos (Chapman et al., 2004). As gastrulation begins, both Wnt5a and Wnt11 are exhibited in Hensen's node (Chapman et al., 2004; Eisenberg et al., 1997). During early gastrulation within the chick, Wnt3, Wnt3a, Wnt4, Wnt5a, Wnt5b, and Wnt8 are exhibited by cells within and/or immediately lateral to the primitive streak (Baranski et al., 2000; Chapman et al., 2004; Esteve et al.,

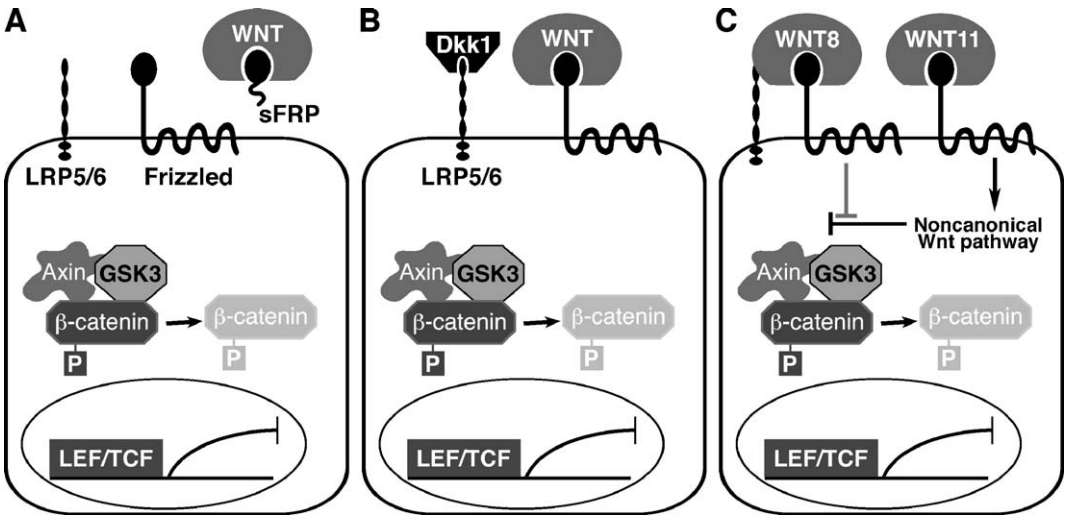

Fig. 3. Multiple types of secreted, extracellular factors can block canonical Wnt signal transduction. (A) sFRPs competitively bind to Wnt proteins and thereby prevent Wnt binding to its cell membrane receptor frizzled. (B) Dkk protein binding to LRP5/6 interferes with the canonical Wnt pathway by preventing Wnt proteins from interacting with this second class of Wnt receptors. (C) Noncanonical Wnt proteins (e.g., Wnt11) can block canonical Wnt signals (represented in the diagram by Wnt8) by downstream signal transduction events. Although the molecular details that account for this suppression have not been well characterized, the activity of noncanonical Wnt signaling is known to prevent the inactivation of GSK3 by canonical Wnts.

2000; Hume and Dodd, 1993). Similar patterns of expression in the mouse have been confirmed for Wnt3, Wnt3a, Wnt5a, and Wnt8 (Bouillet et al., 1996; Kispert et al., 1996; Liu et al., 1999; Takada et al., 1994; Yamaguchi et al., 1999). At the same stages, several Wnts are exhibited in mesodermal domains overlapping with the heart-forming fields. Studies in the chick, quail, and mouse have confirmed that Wnt11 is either expressed within or in close proximity to the precardiac mesoderm (Chapman et al., 2004; Eisenberg et al., 1997; Kispert et al., 1996). In addition, studies in the mouse have demonstrated the precardiac mesodermal expression of both Wnt2 and Wnt2b (Monkley et al., 1996; Zakin et al., 1998).

Table 1
Expression of Wnts and Wnt inhibitors during early gastrulation[a]

Embryonic location	Frog	Chick	Mouse
Organizer[b]	Wnt11, sFRP2, sFRP3 crescent, Dkk1	Wnt5a, Wnt11, Dkk1	Wnt5a, Wnt11
Primitive streak (marginal zone)[†]	Wnt8a, Wnt11, sizzled	Wnt3, Wnt3a, Wnt4, Wnt5a, Wnt5b, Wnt8a, Dkk1	Wnt3, Wnt3a, Wnt5a, Wnt8
Precardiac mesoderm	Wnt11	Wnt11, sFRP3	Wnt2, Wnt2b, Wnt11, sFRP3
Ectoderm or endoderm adjacent to precardiac mesoderm		sFRP2, sFRP3	sFRP3
Anterior to precardiac mesoderm		sFRP1, crescent, Dkk1	

[a] Quick guide to known expression domains of Wnts and their inhibitors at the stages when cardiogenesis is initiated.
[b] The term organizer refers to the Spemann Organizer in the frog, Hensen's node in the chick, and the node in the mouse.
[†] The marginal zone of the frog gastrula is the equivalent structure to the primitive streak in the chick and mouse.

During these stages when the precardiac mesoderm becomes specified, the embryo is marked by the display of several Wnt inhibitors. Both sFRP1 and crescent are expressed in the rostral portion of the early chick gastrula, with the former exhibited in all three germ layers and the latter molecule restricted to the endoderm (Chapman et al., 2004; Esteve et al., 2000; Marvin et al., 2001). Studies in the mouse and chick indicate that sFRP3 expression is exhibited in all three germ layers and overlaps with the heart-forming fields (Baranski et al., 2000; Duprez et al., 1999; Hoang et al., 1998). Also present at these stages is sFRP2, which is exhibited in a broad ectodermal domain overlapping with the heart-forming fields (Chapman et al., 2004; Terry et al., 2000). Dkk1 is highly expressed in Hensen's node, with lower levels throughout the primitive streak and in the rostral endoderm (Chapman et al., 2004; Terry et al., 2000).

A similar pattern of Wnt expression is exhibited during early development of the frog. Xwnt5a, Xwnt8b, and Xwnt11 have been identified as maternally transcribed molecules, whose expression remains detectable during pregastrula stages (Christian and Moon, 1993; Ku and Melton, 1993; Moon et al., 1993; Schroeder et al., 1999). As zygotic transcription commences at the mid-blastula transition, the expression patterns of Wnts and Wnt inhibitors undergo major changes. At the onset of gastrulation, expression of Xwnt11 becomes pronounced in the dorsal marginal zone (DMZ), at the Spemann Organizer, and extending outward to overlap with the bilateral heart-forming fields. As gastrulation proceeds and involution progresses toward the ventral side of the embryo, so does the Xwnt11 domain, as it forms a complete ring around the marginal zone circumference (Ku and Melton, 1993; Tada and Smith, 2000). Xwnt8a expression is first displayed in the late blastulae. Like Xwnt11, Xwnt8a is also displayed along the marginal zone at early gastrulation—but Xwnt8a is exhibited

only in the ventral and lateral sides as this molecule is excluded from the DMZ. By early neurulation, Xwnt8a is exclusively localized to the ventral side of the embryo (Christian and Moon, 1993; Smith and Harland, 1991). Several Wnt inhibitors are known to reside within and lateral to the Spemann Organizer, including crescent, sFRP2, sFRP3, and Dkk1 (Glinka et al., 1998; Pera and De Robertis, 2000; Shibata et al., 2000). In contrast, expression of sizzled, which is another member of the sFRP family, is restricted to the ventral marginal zone (VMZ) (Pera and De Robertis, 2000).

Expression of Wnts and Wnt inhibitors in the developing heart

The expression of both Wnts and Wnt inhibitors continues to be a feature of cardiogenic tissue as it forms into a contractile tube and later remodels into a multi-chambered structure (Table 2). Expression analysis of chick and mouse embryos has indicated that Wnt2b, Wnt5a, Wnt6, Wnt7a, Wnt8a, Wnt9a, Wnt11, sFRP1, sFRP2, and sFRP3 exhibit various distribution patterns in the mid-gestational heart. When the tubular heart initially forms, low levels of expression of Wnt8a, Wnt11, and sFRP1 can be detected in the emerging three-dimensional structure (Jaspard et al., 2000; Kispert et al., 1996). As morphogenesis continues, upregulation of both Wnt8a and sFRP1 was observed in the myocardium of the common ventricular and atrial chambers (Jaspard et al., 2000), while Wnt5a and sFRP3 transcript appear in the ventricle (Baranski et al., 2000). At the same stages, Wnt11 distributes to two distinct locations as it becomes prominently displayed in the outflow tract myocardium (Cai et al., 2003; Phillips et al., 2005) and at low levels in the ventricle (Christiansen et al., 1995)—with the latter appearing to be associated with primitive conduction tissue (Bond et al., 2003). Also associated with the developing conduction tissue is expression of Wnt7a (Bond et al., 2003) and possibly sFRP2, which localizes to the trabeculae of the common ventricle (Ladher et al., 2000). Besides Wnt11, the outflow tract of the tubular heart becomes marked by expression of Wnt5a, Wnt6, Wnt7a, Wnt9a, and sFRP3 (Bond et al., 2003; Eisenberg and Eisenberg, 2002; Ladher et al., 2000; Person et al., 2005; Rodriguez-Niedenfuhr et al., 2003; Schubert et al., 2002). In addition, Wnt2b, Wnt6, Wnt9a, and sFRP3 are also exhibited in the atrioventricular (AV) region of the heart (Eisenberg and Eisenberg, 2002; Ladher et al., 2000; Schubert et al., 2002). Although beyond the scope of this review, the conspicuous expression of multiple Wnts in both the outflow tract and AV canal suggests an important role in cushion tissue development—a proposition supported by several functional studies (Gitler et al., 2003; Liebner et al., 2004). Of greater relevance to this review is the display of Wnt2b, Wnt5a, Wnt6, sFRP3, and Dkk1 in the pharyngeal arches (Baranski et al., 2000; Hoang et al., 1998; Jasoni et al., 1999; Monaghan et al., 1999; Rodriguez-Niedenfuhr et al., 2003; Schubert et al., 2002; Yamaguchi et al., 1999), which is the location of the outflow tract primordium referred to as the anterior or secondary heart field (Eisenberg and Markwald, 2004; Eisenberg et al., 2005). The molecular expression pattern within the pharyngeal arches may indicate that the morphogenesis of the primary heart-forming fields and anterior heart fields shares a common requirement for regulated Wnt signaling.

The distribution of Wnts and their inhibitors in the frog heart has not been as well characterized. The Wnt gene whose expression in the developing frog heart has been best described is Xwnt11-R, which is exhibited throughout the ventricular and atrial myocardium (Garriock et al., 2005). With the recent discovery of Xwnt11-R, a hypothesis was proposed that this molecule, and not the previously described frog Xwnt11, represented the actual ortholog of the individual Wnt11 gene present in the bird and mammalian genome (Garriock et al., 2005). Yet, the Wnt nomenclature is based on the recognition that closely related Wnt genes can be classified according to paralog groups. In the mammalian genome, paralog pairs exist for Wnt2, Wnt3, Wnt5, Wnt7, Wnt9, and Wnt10 (although not Wnt11). Among aquatic vertebrates, there are several instances where paralog groups of Wnt genes have additional members not found in the genome of their terrestrial counterparts. For example, the Wnt4a and Wnt4b paralog pair in fish is orthologous to the single Wnt4 gene found in higher vertebrates. Furthermore, in the frog, there are three closely related Wnt7 genes, as compared to the Wnt7a,b pair that is present in the bird and mammalian genome. The sharing of unique sequence motifs among chick Wnt11, mouse Wnt11, *Xenopus* Xwnt11, and Xwnt11-R suggests that the two frog genes are paralogs. Further support for the paralogous relationship of the two *Xenopus* Wnt11 molecules is provided by the expression patterns of the bird and mouse Wnt11, which correspond to the composite distributions of Xwnt11 and Xwnt11-R genes (Garriock et al., 2005; Ku and Melton, 1993; Tada and Smith,

Table 2
Expression of Wnts and Wnt inhibitors in the developing heart[a]

Molecule	Mouse
Wnt2b	Outflow tract primordium[b]; atrioventricular region of the tubular heart
Wnt5a	Outflow tract primordium; outflow tract of the tubular hear; common ventricle
Wnt6	Outflow tract primordium; outflow tract and atrioventricular regions of the tubular heart
Wnt7a	Primitive conduction tissue; outflow tract of the tubular heart
Wnt8a	Newly formed primary heart tube; common ventricular and atrial chambers
Wnt9a	Outflow tract and atrioventricular regions of the tubular heart
Wnt11	Newly formed primary heart tube; outflow tract myocardium; primitive conduction tissue
sFRP1	Newly formed primary heart tube; common ventricular and atrial chambers
sFRP2	Trabeculae of the common ventricle
sFRP3	Outflow tract primordium; outflow tract of the tubular heart; common ventricle
Dkk1	Outflow tract primordium; outflow tract of the tubular heart; common ventricle

[a] Quick guide to known expression domains of Wnts and their inhibitors in the developing heart at mid-gestational stages of the chick and mouse embryo.
[b] Located in the pharyngeal mesoderm and referred to as the anterior or secondary heart field.

2000). Xwnt11 is exhibited at early gastrulation in proximity to the precardiac mesoderm but is absent from the developing heart. In contrast, Xwnt11-R is displayed in the developing heart but is not expressed at earlier time points. As mentioned above, studies of the embryonic quail (Eisenberg et al., 1997), chick (Chapman et al., 2004), and mouse (Kispert et al., 1996) have demonstrated that Wnt11 is expressed during early gastrulation. Interestingly, there is a greater divergence between the expression patterns of Xwnt11-R and bird/mouse Wnt11 as the former displays a much broader distribution within the developing heart. Despite this caveat, both sequence and expression data provide strong evidence for an alternative hypothesis that the two *Xenopus* Wnt11 genes comprise a paralog pair.

Functional studies examining the influence of Wnt signaling on heart formation

The first evidence that Wnt signaling plays an important role in cardiac tissue formation came from studies with *Drosophila* (Park et al., 1996). In that species, the formation of the dorsal vessel, which is a primitive antecedent of the vertebrate heart, is dependent on the expression of the *Drosophila* Wnt1 ortholog *wingless*. The initial demonstration that Wnt signaling may also influence vertebrate cardiogenesis involved experimentation with embryonic quail tissue explants. In that study (Eisenberg and Eisenberg, 1999), tissue was harvested from the posterior end of the early avian gastrula – which is an embryonic region that does not contribute to heart formation in situ – and cultured in the presence or absence of Wnt11. The exposure to Wnt11 provoked the posterior tissue explants to form small areas of cardiac tissue. Subsequent studies with chick explants have indicated that Dkk1 and crescent possess similar abilities to promote cardiogenesis (Eisenberg and Eisenberg, 2004; Marvin et al., 2001).

An animal model that has loomed large in codifying current thinking about the role of Wnt signal transduction in heart formation is the frog embryo. The frog heart arises from mesoderm derived from the DMZ, while mesoderm tissue derived from the VMZ does not provide any cellular material to the developing heart (Mohun et al., 2003). Experiments were performed where RNA was injected into the ventral blastomeres at the 4-cell stage, and after letting the embryos develop further to early gastrulation stages, the VMZ was subsequently removed from the embryo and cultured. Initial experimentation with this assay indicated that injected Dkk1 and crescent RNA was able to induce myocardial tissue formation from the putative noncardiogenic VMZ tissue (Schneider and Mercola, 2001). Since Dkk1 and crescent have been viewed strictly as Wnt inhibitors, the reported activity of these molecules as inducers of cardiogenesis was interpreted as a demonstration that Wnt inhibition is an absolute requirement for heart development (Marvin et al., 2001; Olson, 2001; Schneider and Mercola, 2001). This idea that Wnt inhibition is the molecular impetus driving the formation of the vertebrate heart (Fig. 4A) has maintained a lot of traction in cardiovascular developmental biology and is still widely reported (Brott and Sokol, 2005; Garriock et al., 2005).

Subsequent to the reports on the cardiogenic activities of Dkk1 and crescent, a similar study in the frog embryo showed that Wnt11 is also able to promote the myocardial differentiation of VMZ tissue (Pandur et al., 2002). In this case, the injection of Wnt11 RNA into the ventral blastomeres promoted cardiac tissue formation in VMZ explants by triggering a noncanonical, JNK-mediated pathway. In conjunction with the earlier example of Wnt11 stimulation of cardiomyocyte differentiation in avian tissue explants (Eisenberg and Eisenberg, 1999), a new paradigm emerged (Fig. 4B) where heart formation was not simply the result of Wnt inhibition, but more specifically involved the suppression of 'canonical' Wnt

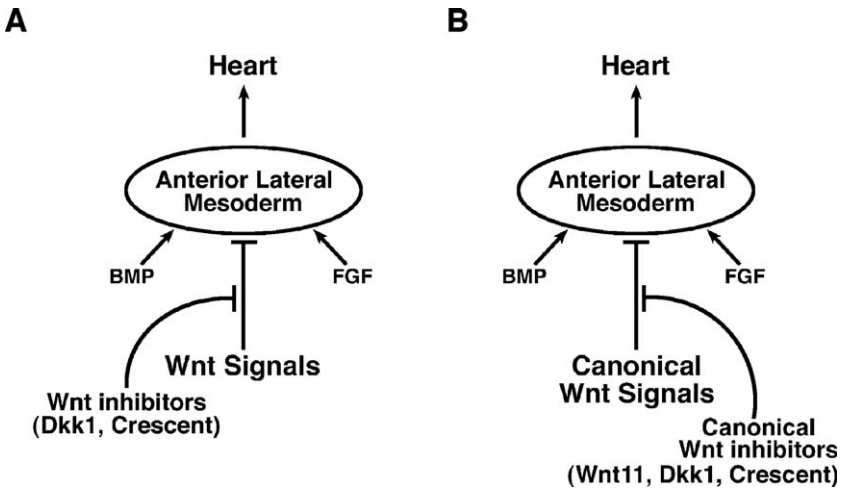

Fig. 4. Current views on the molecular signals that induce the formation of the vertebrate heart. (A) Reports that both Dkk1 and crescent can promote cardiac tissue formation prompted the model that inhibition of Wnt signaling is a necessary condition for allowing the heart to form from anterior lateral mesoderm (Marvin et al., 2001; Olson, 2001; Schneider and Mercola, 2001). (B) During the past few years, the conception of WNT signal transduction has evolved considerably, and it is now accepted that multiple WNT signaling pathways exist. Accordingly, models of heart induction have also evolved and now characterize the requirement of WNT signal inhibition as specifically involving the suppression of canonical Wnt signal transduction (Foley and Mercola, 2004; Pandur, 2005; Solloway and Harvey, 2003). Other molecules that are commonly cited as important players of heart induction are members of the BMP and FGF family (Brand, 2003; Pandur, 2005).

signaling (Pandur et al., 2002). In this model, heart formation is induced by a multitude of molecules that interfere with canonical Wnt signaling (Foley and Mercola, 2004; Pandur, 2005; Solloway and Harvey, 2003; van den Hoff et al., 2004), either by directly inhibiting the ability of canonical Wnts to bind to the cell surface and/or by noncanonical Wnt inhibition of downstream targets of the canonical pathway (e.g., β-catenin). Data from several other studies were consistent with this new model as Wnt11 was reported to stimulate cardiomyocyte differentiation of various stem cell populations (Koyanagi et al., 2005; Pandur et al., 2002; Terami et al., 2004). In addition, the preferential ablation of the β-catenin gene in the developing mouse endoderm produced embryos with multiple cardiac structures (Lickert et al., 2002). Thus, the loss of β-catenin expression may have specifically prompted noncardiac progenitors to form cardiac tissue.

Is the canonical Wnt inhibition model of heart formation consistent with molecular expression in the early embryo?

The current paradigm of heart formation is that inhibition of canonical Wnt signal transduction is an absolute requirement for specifying myocardial cell fate (Brand, 2003; Foley and Mercola, 2004; Solloway and Harvey, 2003; van den Hoff et al., 2004). According to this model, heart formation does not result solely from Wnt inhibition as other signals are also thought to be necessary for the inducing cardiac tissue formation—most notably, FGF and BMP signals. Yet, the subtext of this model is that canonical Wnts are antithetical to the expression of a myocardial phenotype. One question that may be proposed is whether this paradigm is consistent with the known expression of Wnts in the early embryo and developing heart.

In critiquing the function of Wnts in cardiogenesis, let us start with the obvious—that the pattern of expression of canonical Wnts, noncanonical Wnts, and various classes of Wnt inhibitors is very complex. Moreover, it needs to be kept in mind that these genes encode for secreted proteins. At the time heart formation is initiated, the embryo is undergoing very rapid growth and remodeling. That cells within the early embryo are highly proliferative and on the move undoubtedly impacts the distribution of Wnt, sFRP, and Dkk proteins in the emerging organism. For example, in the early avian embryo, the Wnt11 gene is expressed by mesodermal cells that are spreading laterally from Hensen's node and/or the primitive streak (Chapman et al., 2004; Eisenberg et al., 1997). How far anterior and posterior Wnt11 protein spreads from the gene expression domain, and how this may be reflected in a protein concentration gradient, is impossible to predict from the pattern elucidated from in situ hybridization analysis. An additional consideration is the distributions of frizzled proteins, which are cell membrane receptors that can impact the signaling properties of Wnts—including whether Wnt5a class molecules trigger canonical or noncanonical Wnt pathways. Thus, in the absence of mathematical modeling that would compile all this molecular information, it is not easy to fully comprehend how the distributions of each of these various types of molecules may influence the functional activities that regulate cardiogenesis.

The expression of various Wnts in the developing heart makes it very difficult to build the case that canonical Wnt signaling is at variance with a myocardial cell fate. This is most dramatically demonstrated by the broad distribution within the developing myocardium of Wnt8a (Jaspard et al., 2000), which along with Wnt1 is the quintessential canonical Wnt (Christian and Moon, 1993; Sokol et al., 1991; Torres et al., 1996). There is also the expression of Wnt2b, Wnt6, Wnt7a, and Wnt9a – which are all molecules that can stimulate canonical Wnt signal transduction (Karasawa et al., 2002; Linker et al., 2005; Person et al., 2005; Wong et al., 1994) – in the AV canal, outflow tract, and the anterior heart field of the pharyngeal arches (Bond et al., 2003; Eisenberg and Eisenberg, 2002; Jasoni et al., 1999; Person et al., 2005; Rodriguez-Niedenfuhr et al., 2003; Schubert et al., 2002). Although evidence indicates that the importance of some of these signals may be of greater relevance to regulating cardiac cushion development (Gitler et al., 2003; Liebner et al., 2004), there are also functional data indicating that canonical Wnt signal transduction can have a positive impact on myocardial differentiation and phenotype (Ai et al., 2000; Naito et al., 2005; Nakamura et al., 2003). Thus, it appears that Wnts promote the development of both myocardial and nonmuscle tissues of the heart.

Does Dkk1 and crescent function solely as inhibitors of canonical Wnt inhibition?

The assertion that there is an antithetical relationship between canonical Wnt signaling and myocardial cell fate is based primarily on the identification of Dkk1 and crescent as cardiac 'inducers.' These two molecules have been dubbed as canonical Wnt inhibitors, and, thus, it is accepted that Wnt inhibition is their sole function. Yet, that characterization of the functional properties of these two molecules may not be totally accurate. As shown with Wnt11, JNK activation is associated with cardiac tissue formation in VMZ explants following the ventral injections of Dkk1 or crescent RNA into the frog blastula (Pandur et al., 2002). In other words, Dkk1 and crescent triggering of signal transduction pathways associated with noncanonical Wnts may be the stimulus that accounts for the cardiogenic activities of these molecules. It also should be considered that, if inhibition of canonical Wnts is the sole story of how these molecules induce cardiogenesis, then sFRP3 should be a great cardiac inducer. However, sFRP3, which is the most definitive and reliable canonical Wnt inhibitor yet characterized (Lin et al., 1997; Wang et al., 1997a,b), has shown little ability to promote cardiac tissue formation (Schneider and Mercola, 2001).

The ability of Dkk1 to inhibit canonical Wnt signaling is dependent on the co-expression of kremen cell membrane proteins (Davidson et al., 2002; Mao et al., 2002). Interestingly, in the absence of kremen expression, another member of the Dkk family, Dkk2, can enhance canonical Wnt signaling (Mao and Niehrs, 2003). Whether Dkk1 is also

capable of enhancing Wnt activity under certain conditions has not been actively investigated. Yet, there is an intriguing result from the initial characterization of Dkk1 that showed that this molecule can rescue ventralized UV-treated frog embryos (Glinka et al., 1998)—which is a property that Dkk1 shares with canonical Wnts (Sokol et al., 1991). There are also data to indicate that sFRPs may have the ability to potentiate canonical Wnt responses (Bafico et al., 1999; Kawano and Kypta, 2003). Crescent may be the least characterized member of the sFRP family in regard to its Wnt inhibitory activities. In addition, crescent may possess functional activities that are unique among sFRP proteins. The injection of crescent RNA into frog embryo promotes cyclopia, which was a result not observed with other sFRP molecules (Pera and De Robertis, 2000). These observations on the properties of Dkk1 and crescent are not made to dismiss their Wnt inhibitory activities as possible mechanisms for initiating cardiogenesis. Instead, the purpose here is to point out that a characterization of these molecules simply as Wnt inhibitors may not do justice to their full range of activities, which could lead to a less than complete understanding of their role in promoting heart formation.

Wnt regulation of tissue remodeling in the embryo—an alternative model of heart formation

On the face of it, the report that multiple hearts formed in mice with a conditional knockout of β-catenin would appear to be the final piece that confirms the model that the heart forms in response to Wnt inhibition (Lickert et al., 2002). Yet, the extreme abnormalities of these genetically altered mouse embryos suggest an alternative diagnosis. It is obvious that tissue movements in these mice were severely altered, which might have led to the maldistribution of organ rudiments, such as the heart. Interestingly, Wnt signaling has been well characterized for regulating tissue movements. The best described examples involve Wnt5a and Wnt11 regulation of convergent/extension movements in the early zebrafish and *Xenopus* embryo (Heisenberg et al., 2000; Solnica-Krezel, 2001; Tada and Smith, 2000). More recently, studies in zebrafish have shown that Wnt4a, Wnt11, and Wnt11-R regulate midline convergence of organ primordia, including the bilateral cardiogenic fields (Matsui et al., 2005). As described above, ectopic expression of crescent causes cyclopia (Pera and De Robertis, 2000), where midline structures fail to bifurcate and spread laterally. Wnts are also known to regulate epithelial–mesenchymal transformations, tissue epithelialization, tissue polarity, and organ segmentation. Thus, Wnt signal transduction appears to have major influence in regulating basic morphological events that underlie tissue and organ development (Matsui et al., 2005; Ulrich et al., 2005). In regard to cardiogenesis, there are several morphological events during heart development that are candidates for regulated Wnt signaling playing a major role, including the formation of the mesoderm by gastrulation, coalescing of newly formed mesoderm to form the heart-forming fields, movement of the bilateral precardiac mesodermal fields toward the ventral midline, subsequent fusion of the heart-forming fields to form the tubular heart, segmentation of the developing heart, extension of the trabeculae, movement of pharyngeal mesoderm into the primitive heart, seeding of endocardial cells into the cardiac cushions, the condensation of cushion cells to form

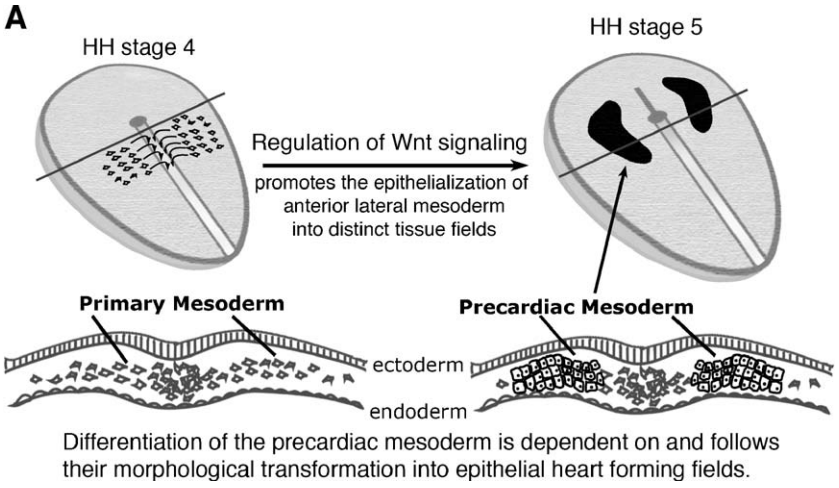

Fig. 5. Alternative model for understanding the role WNT regulation plays in heart development. Since Wnts are known to play major roles in regulating basic morphological events that underlie tissue and organ development, we suggest that Wnts are not necessarily involved in specifying cardiac cell fate per se. Instead, cardiac cell fate is dependent on and follows the morphological regulation of the emerging tissue. This model is illustrated in this diagram by the differentiation of the precardiac mesoderm, which comprises bilateral fields of epithelialized tissue. We propose that regulated Wnt activity influences the ability of newly gastrulated cells to coalesce into distinct tissue fields. The subsequent cardiac differentiation of this mesoderm tissue is a consequence of this crucial first step in cardiac organogenesis. In addition, there are several morphological events during heart development that are candidates for Wnt regulation playing a major role, including the movement of the bilateral precardiac mesodermal fields toward the ventral midline, subsequent fusion of the heart-forming fields to form the tubular heart, segmentation of the developing heart, extension of the trabeculae, movement of pharyngeal mesoderm into the primitive heart, seeding of endocardial cells into the cardiac cushions, the condensation of cushion tissue to form valves, segregation of conduction tissue from the working myocardium, and epicardial cell migration and integration into the heart. It is hypothesized that some episodes in cardiac morphogenesis may require canonical Wnt signal to be suppressed, while others may require activation of this pathway.

valvular tissue, segregation of conduction tissue from the working myocardium, and epicardial cell migration and integration into the heart. In this model (Fig. 5), Wnt11, Dkk1, and crescent would not be involved in specifying cardiac cell fate per se. Instead, cardiac cell fate is dependent on and follows the morphological regulation of the emerging tissue. This is exemplified by the differentiation of the precardiac mesoderm, which occurs subsequent to the coalescence of newly gastrulated cells into distinct tissue fields. Some episodes in cardiac morphogenesis may require canonical Wnt signal to be suppressed, while others may require activation of this pathway. Here, this model takes a page from the recent findings in regard to BMP signaling, where cardiogenesis is promoted by either BMP activation or inhibition, depending on the embryonic stage the signal is provided (Yuasa et al., 2005). While Wnt signaling may not play significant roles in all the developmental events mentioned above, the multifaceted and continual expression of Wnts and Wnt inhibitors in the developing heart certainly suggests that these molecules are major players throughout cardiac morphogenesis.

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References

Ai, Z.W., Fischer, A., Spray, D.C., Brown, A.M.C., Fishman, G.I., 2000. Wnt-1 regulation of connexin43 in cardiac myocytes. J. Clin. Invest. 105, 161–171.

Bafico, A., Gazit, A., Pramila, T., Finch, P.W., Yaniv, A., Aaronson, S.A., 1999. Interaction of frizzled related protein (FRP) with Wnt ligands and the frizzled receptor suggests alternative mechanisms for FRP inhibition of Wnt signaling. J. Biol. Chem. 274, 16180–16187.

Bafico, A., Liu, G., Yaniv, A., Gazit, A., Aaronson, S.A., 2001. Novel mechanism of Wnt signalling inhibition mediated by Dickkopf-1 interaction with LRP6/Arrow. Nat. Cell Biol. 3, 683–686.

Baranski, M., Berdougo, E., Sandler, J.S., Darnell, D.K., Burrus, L.W., 2000. The dynamic expression pattern of frzb-1 suggests multiple roles in chick development. Dev. Biol. 217, 25–41.

Bejsovec, A., 2005. Wnt pathway activation: new relations and locations. Cell 120, 11–14.

Bond, J., Sedmera, D., Jourdan, J., Zhang, Y., Eisenberg, C.A., Eisenberg, L.M., Gourdie, R.G., 2003. Wnt11 and Wnt7a are up-regulated in association with differentiation of cardiac conduction cells in vitro and in vivo. Dev. Dyn. 227, 536–543.

Bouillet, P., Ouladabdelghani, M., Ward, S.J., Bronner, S., Chambon, P., Dolle, P., 1996. A new mouse member of the WNT gene family, mWNT-8, is expressed during early embryogenesis and is ectopically induced by retinoic acid. Mech. Dev. 58, 141–152.

Brand, T., 2003. Heart development: molecular insights into cardiac specification and early morphogenesis. Dev. Biol. 258, 1–19.

Brott, B.K., Sokol, S.Y., 2005. A vertebrate homolog of the cell cycle regulator Dbf4 is an inhibitor of Wnt signaling required for heart development. Dev. Cell 8, 703–715.

Cai, C.-L., Liang, X., Shi, Y., Chu, P.-H., Pfaff, S.L., Chen, J., Evans, S., 2003. Isl1 identifies a cardiac progenitor population that proliferates prior to differentiation and contributes a majority of cells to the heart. Dev. Cell 5, 877–889.

Chapman, S.C., Brown, R., Lees, L., Schoenwolf, G.C., Lumsden, A., 2004. Expression analysis of chick Wnt and frizzled genes and selected inhibitors in early chick patterning. Dev. Dyn. 229, 668–676.

Christian, J.L., Moon, R.T., 1993. Interactions between Xwnt-8 and Spemann organizer signaling pathways generate dorsoventral pattern in the embryonic mesoderm of *Xenopus*. Genes Dev. 7, 13–28.

Christiansen, J.H., Dennis, C.L., Wicking, C.A., Monkley, S.J., Wilkinson, D.G., Wainwright, B.J., 1995. Murine wnt-11 and wnt-12 have temporally and spatially restricted expression patterns during embryonic development. Mech. Dev. 51, 341–350.

Davidson, G., Mao, B., del Barco Barrantes, I., Niehrs, C., 2002. Kremen proteins interact with Dickkopf1 to regulate anteroposterior CNS patterning. Development 129, 5587–5596.

Du, S.J., Purcell, S.M., Christian, J.L., McGrew, L.L., Moon, R.T., 1995. Identification of distinct classes and functional domains of *wnts* through expression of wild-type and chimeric proteins in *Xenopus* embryos. Mol. Cell. Biol. 15, 2625–2634.

Duprez, D., Leyns, L., Bonnin, M.-A., Lapointe, F., Etchevers, H., Robertis, E.M.D., Douarin, N.L., 1999. Expression of Frzb-1 during chick development. Mech. Dev. 89, 179–183.

Eisenberg, C.A., Eisenberg, L.M., 1999. WNT11 promotes cardiac tissue formation of early mesoderm. Dev. Dyn. 216, 45–58.

Eisenberg, L.M., Eisenberg, C.A., 2002. Onset of a cardiac phenotype in the early embryo. In: Dube, D.K. (Ed.), Cardiovascular Molecular Morphogenesis: Myofibrillogenesis. Springer Verlag, New York, NY, pp. 181–205.

Eisenberg, L.M., Eisenberg, C.A., 2004. An in vitro analysis of myocardial potential indicates that phenotypic plasticity is an innate property of early embryonic tissue. Stem Cells Dev. 13, 614–624.

Eisenberg, L.M., Markwald, R.R., 2004. Cellular recruitment and the development of the myocardium. Dev. Biol. 274, 225–232.

Eisenberg, C.A., Gourdie, R.G., Eisenberg, L.M., 1997. *Wnt11* is expressed in early avian mesoderm and required for the differentiation of the quail mesoderm cell line QCE6. Development 124, 525–536.

Eisenberg, L.M., Moreno, R., Markwald, R.R., 2005. Multiple stem cell populations contribute to the formation of the myocardium. Ann. N. Y. Acad. Sci. 1047, 38–49.

Esteve, P., Morcillo, J., Bovolenta, P., 2000. Early and dynamic expression of cSfrp1 during chick embryo development. Mech. Dev. 97, 217–221.

Fanto, M., McNeill, H., 2004. Planar polarity from flies to vertebrates. J. Cell Sci. 117, 527–533.

Foley, A., Mercola, M., 2004. Heart induction: embryology to cardiomyocyte regeneration. Trends Cardiovasc. Med. 14, 121–125.

Garriock, R.J., D'Agostino, S.L., Pilcher, K.C., Krieg, P.A., 2005. Wnt11-R, a protein closely related to mammalian Wnt11, is required for heart morphogenesis in *Xenopus*. Dev. Biol. 279, 179–192.

Gitler, A.D., Lu, M.M., Jiang, Y.Q., Epstein, J.A., Gruber, P.J., 2003. Molecular markers of cardiac endocardial cushion development. Dev. Dyn. 228, 643–650.

Glinka, A., Wu, W., Delius, H., Monaghan, A.P., Blumenstock, C., Niehrs, C., 1998. Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. Nature 391, 357–362.

He, X., Saint-Jeannet, J.-P., Wang, Y., Nathans, J., Dawid, I., Varmus, H., 1997. A member of the Frizzled protein family mediating axis induction by Wnt-5A. Science 275, 1652–1654.

He, X., Semenov, M., Tamai, K., Zeng, X., 2004. LDL receptor-related proteins 5 and 6 in Wnt/beta-catenin signaling: arrows point the way. Development 131, 1663–1677.

Heisenberg, C.P., Tada, M., Rauch, G.J., Saude, L., Concha, M.L., Geisler, R., Stemple, D.L., Smith, J.C., Wilson, S.W., 2000. Silberblick/Wnt-11 mediates convergent extension movements during zebrafish gastrulation. Nature 405, 76–81.

Hoang, B.H., Thomas, J.T., Abdulkarim, F.W., Correia, K.M., Conlon, R.A., Luyten, F.P., Ballock, R.T., 1998. Expression pattern of two frizzled-related genes, frzb-1 and sfrp-1, during mouse embryogenesis suggests a role for modulating action of Wnt family members. Dev. Dyn. 212, 364–372.

Huang, H.C., Klein, P.S., 2004. The Frizzled family: receptors for multiple signal transduction pathways. Genome Biol. 5, 234.

Hume, C.R., Dodd, J., 1993. *Cwnt-8C*: a novel Wnt gene with a potential role in primitive streak formation and hindbrain organization. Development 119, 1147–1160.

Jasoni, C., Hendrickson, A., Roelink, H., 1999. Analysis of chicken Wnt-13 expression demonstrates coincidence with cell division in the developing eye and is consistent with a role in induction. Dev. Dyn. 215, 215–224.

Jaspard, B., Couffinhal, T., Dufourcq, P., Moreau, C., Duplaa, C., 2000. Expression pattern of mouse sFRP-1 and mWnt-8 gene during heart morphogenesis. Mech. Dev. 90, 263–267.

Karasawa, T., Yokokura, H., Kitajewski, J., Lombroso, P.J., 2002. Frizzled-9 is activated by Wnt-2 and functions in Wnt/beta-catenin signaling. J. Biol. Chem. 277, 37479–37486.

Kawano, Y., Kypta, R., 2003. Secreted antagonists of the Wnt signalling pathway. J. Cell Sci. 116, 2627–2634.

Keller, R., 2002. Shaping the vertebrate body plan by polarized embryonic cell movements. Science 298, 1950–1954.

Kispert, A., Vainio, S., Shen, L., Rowitch, D.H., McMahon, A.P., 1996. Proteoglycans are required for maintenance of Wnt-11 expression in the ureter tips. Development 122, 3627–3637.

Koyanagi, M., Haendeler, J., Badorff, C., Brandes, R.P., Hoffmann, J., Pandur, P., Zeiher, A.M., Kühl, M., Dimmeler, S., 2005. Non-canonical Wnt signaling enhances differentiation of human circulating progenitor cells to cardiomyogenic cells. J. Biol. Chem. 280, 16838–16842.

Ku, M., Melton, D., 1993. *Xwnt-11*: a maternally expressed *Xenopus* Wnt gene. Development 119, 1161–1173.

Kühl, M., 2004. The WNT/calcium pathway: biochemical mediators, tools and future requirements. Front. Biosci. 9, 967–974.

Kühl, M., Geis, K., Sheldahl, L.C., Pukrop, T., Moon, R.T., Wedlich, D., 2001. Antagonistic regulation of convergent extension movements in *Xenopus* by Wnt/β-catenin and Wnt/Ca^{2+} signaling. Mech. Dev. 106, 61–76.

Ladher, R.K., Church, V.L., Allen, S., Robson, L., Abdelfattah, A., Brown, N.A., Hattersley, G., Rosen, V., Luyten, F.P., Dale, L., Francis-West, P.H., 2000. Cloning and expression of the Wnt antagonists Sfrp-2 and Frzb during chick development. Dev. Biol. 218, 183–198.

Lickert, H., Kutsch, S., Kanzler, B., Tamai, Y., Taketo, M.M., Kemler, R., 2002. Formation of multiple hearts in mice following deletion of β-catenin in the embryonic endoderm. Dev. Cell 3, 171–181.

Liebner, S., Cattelino, A., Gallini, R., Rudini, N., Iurlaro, M., Piccolo, S., Dejana, E., 2004. Beta-catenin is required for endothelial–mesenchymal transformation during heart cushion development in the mouse. J. Cell Biol. 166, 359–367.

Lin, K.M., Wang, S.W., Julius, M.A., Kitajewski, J., Moos, M., Luyten, F.P., 1997. The cysteine-rich frizzled domain of Frzb-1 is required and sufficient for modulation of Wnt signaling. Proc. Natl. Acad. Sci. U. S. A. 94, 11196–11200.

Linker, C., Lesbros, C., Gros, J., Burrus, L.W., Rawls, A., Marcelle, C., 2005. ß-catenin-dependent Wnt signalling controls the epithelial organisation of somites through the activation of paraxis. Development 132, 3895–3905.

Liu, P., Wakamiya, M., Shea, M.J., Albrecht, U., Behringer, R.R., Bradley, A., 1999. Requirement for Wnt3 in vertebrate axis formation. Nat. Genet. 22, 361–365.

Logan, C.Y., Nusse, R., 2004. The Wnt signaling pathway in development and disease. Annu. Rev. Cell Dev. Biol. 20, 781–810.

Luo, W., Lin, S.C., 2004. Axin: a master scaffold for multiple signaling pathways. NeuroSignals 13, 99–113.

Lyons, J.P., Mueller, U.W., Ji, H., Everett, C., Fang, X., Hsieh, J.C., Barth, A.M., McCrea, P.D., 2004. Wnt-4 activates the canonical beta-catenin-mediated Wnt pathway and binds Frizzled-6 CRD: functional implications of Wnt/beta-catenin activity in kidney epithelial cells. Exp. Cell Res. 298, 369–387.

Mao, B., Niehrs, C., 2003. Kremen2 modulates Dickkopf2 activity during Wnt/LRP6 signaling. Gene 302, 179–183.

Mao, B., Wu, W., Li, Y., Hoppe, D., Stannek, P., Glinka, A., Niehrs, C., 2001a. LDL-receptor-related protein 6 is a receptor for Dickkopf proteins. Nature 411, 321–325.

Mao, J., Wang, J., Liu, B., Pan, W., Farr III, G., Flynn, C., Yuan, H., Takada, S., Kimelman, D., Li, L., Wu, D., 2001b. Low-density lipoprotein receptor-related Protein-5 binds to axin and regulates the canonical Wnt signalling pathway. Mol. Cell 7, 801–809.

Mao, B., Wu, W., Davidson, G., Marhold, J., Li, M., Mechler, B.M., Delius, H., Hoppe, D., Stannek, P., Walter, C., Glinka, A., Niehrs, C., 2002. Kremen proteins are Dickkopf receptors that regulate Wnt/beta-catenin signalling. Nature 417, 664–667.

Marvin, M.J., Di Rocco, G., Gardiner, A., Bush, S.M., Lassar, A.B., 2001. Inhibition of Wnt activity induces heart formation from posterior mesoderm. Genes Dev. 15, 316–327.

Matsui, T., Raya, A., Kawakami, Y., Callol-Massot, C., Capdevila, J., Rodriguez-Esteban, C., Izpisua Belmonte, J.C., 2005. Noncanonical Wnt signaling regulates midline convergence of organ primordia during zebrafish development. Genes Dev. 19, 164–175.

Maurus, D., Heligon, C., Burger-Schwarzler, A., Brandli, A.W., Kühl, M., 2005. Noncanonical Wnt-4 signaling and EAF2 are required for eye development in *Xenopus laevis*. EMBO J. 24, 1181–1191.

Maye, P., Zheng, J., Li, L., Wu, D., 2004. Multiple mechanisms for Wnt11-mediated repression of the canonical Wnt signaling pathway. J. Biol. Chem. 279, 24659–24665.

Mohun, T., Orford, R., Shang, C., 2003. The origins of cardiac tissue in the amphibian, *Xenopus laevis*. Trends Cardiovasc. Med. 13, 244–248.

Monaghan, A.P., Kioschis, P., Wu, W., Zuniga, A., Bock, D., Poustka, A., Delius, H., Niehrs, C., 1999. *Dickkopf* genes are co-ordinately expressed in mesodermal lineages. Mech. Dev. 87, 45–56.

Monkley, S.J., Delaney, S.J., Pennisi, D.J., Christiansen, J.H., Wainwright, B.J., 1996. Targeted disruption of the Wnt2 gene results in placentation defects. Development 122, 3343–3353.

Moon, R.T., Campbell, R.M., Christian, J.L., McGrew, L.L., Shih, J., Fraser, S., 1993. Xwnt-5A: a maternal Wnt that affects morphogenetic movements after overexpression in embryos of *Xenopus laevis*. Development 119, 97–111.

Moon, R.T., Brown, J.D., Torres, M., 1997. WNTs modulate cell fate and behavior during vertebrate development. Trends Genet. 13, 157–162.

Moon, R.T., Kohn, A.D., De Ferrari, G.V., Kaykas, A., 2004. WNT and beta-catenin signalling: diseases and therapies. Nat. Rev., Genet. 5, 691–701.

Naito, A.T., Akazawa, H., Takano, H., Minamino, T., Nagai, T., Aburatani, H., Komuro, I., 2005. Phosphatidylinositol 3-kinase-Akt pathway plays a critical role in early cardiomyogenesis by regulating canonical Wnt signaling. Circ. Res. 97, 144–151.

Nakamura, T., Sano, M., Songyang, Z., Schneider, M.D., 2003. A Wnt- and ß-catenin-dependent pathway for mammalian cardiac myogenesis. Proc. Natl. Acad. Sci. U. S. A. 100, 5834–5839.

Olson, E.N., 2001. Development—The path to the heart and the road not taken. Science 291, 2327–2328.

Pandur, P., 2005. What does it take to make a heart? Biol. Cell 97, 197–210.

Pandur, P., Läsche, M., Eisenberg, L.M., Kühl, M., 2002. Wnt-11 activation of a non-canonical Wnt signaling pathway is required for cardiogenesis. Nature 418, 636–641.

Park, M., Moon, R.T., 2002. The planar cell-polarity gene stbm regulates cell behaviour and cell fate in vertebrate embryos. Nat. Cell Biol. 4, 20–25.

Park, M., Wu, X., Golden, K., Axelrod, J.D., Bodmer, R., 1996. The Wingless signaling pathway is directly involved in *Drosophila* heart development. Dev. Biol. 177, 104–116.

Pera, E.M., De Robertis, E.M., 2000. A direct screen for secreted proteins in *Xenopus* embryos identifies distinct activities for the Wnt antagonists Crescent and Frzb-1. Mech. Dev. 96, 183–195.

Person, A.D., Garriock, R.J., Krieg, P.A., Runyan, R.B., Klewer, S.E., 2005. Frzb modulates Wnt-9a-mediated ß-catenin signaling during avian atrioventricular cardiac cushion development. Dev. Biol. 278, 35–48.

Phillips, H.M., Murdoch, J.N., Chaudhry, B., Copp, A.J., Henderson, D.J., 2005. Vangl2 acts via RhoA signaling to regulate polarized cell movements during development of the proximal outflow tract. Circ. Res. 96, 292–299.

Rattner, A., Hsieh, J.-C., Smallwood, P.M., Gilbert, D.J., Copeland, N.G., Jenkins, N.A., Nathans, J., 1997. A family of secreted proteins contains homology to the cysteine-rich ligand-binding domain of frizzled receptors. Proc. Natl. Acad. Sci. U. S. A. 94, 2859–2863.

Reya, T., Clevers, H., 2005. Wnt signalling in stem cells and cancer. Nature 434, 843–850.

Rodriguez-Niedenfuhr, M., Dathe, V., Jacob, H.J., Prols, F., Christ, B., 2003. Spatial and temporal pattern of Wnt-6 expression during chick development. Anat. Embryol. 206, 447–451.

Schneider, V.A., Mercola, M., 2001. Wnt antagonism initiates cardiogenesis in *Xenopus laevis*. Genes Dev. 15, 304–315.

Schroeder, K.E., Condic, M.L., Eisenberg, L.M., Yost, H.J., 1999. Spatially regulated translation in embryos: asymmetric expression of maternal Wnt-11 along the dorsal–ventral Wnt-11 along the dorsal–ventral axis in *Xenopus*. Dev. Biol. 214, 288–297.

Schubert, F.R., Mootoosamy, R.C., Walters, E.H., Graham, A., Tumiotto, L., Münsterberg, A.E., Lumsden, A., Dietrich, S., 2002. Wnt6 marks sites of epithelial transformations in the chick embryo. Mech. Dev. 114, 143–148.

Semenov, M.V., Tamai, K., Brott, B.K., Kühl, M., Sokol, S., He, X., 2001. Head inducer Dickkopf-1 is a ligand for Wnt coreceptor LRP6. Curr. Biol. 11, 951–961.

Sheldahl, L.C., Park, M., Malbon, C.C., Moon, R.T., 1999. Protein kinase C is differentially stimulated by Wnt and Frizzled homologs in a G-protein-dependent manner. Curr. Biol. 9, 695–698.

Sheldahl, L.C., Slusarski, D.C., Pandur, P., Miller, J.R., Kühl, M., Moon, R.T., 2003. Dishevelled activates Ca^{2+} flux, PKC, and CamKII in vertebrate embryos. J. Cell Biol. 161, 769–777.

Shibata, M., Ono, H., Hikasa, H., Shinga, J., Taira, M., 2000. *Xenopus* crescent encoding a Frizzled-like domain is expressed in the Spemann organizer and pronephros. Mech. Dev. 96, 243–246.

Skromne, I., Stern, C.D., 2001. Interactions between Wnt and Vg1 signalling pathways initiate primitive streak formation in the chick embryo. Development 128, 2915–2927.

Slusarski, D.C., Yang-Snyder, J., Busa, W.B., Moon, R.T., 1997. Modulation of embryonic intracellular Ca^{2+} signaling by Wnt-5A. Dev. Biol. 182, 114–120.

Smith, W.C., Harland, R.M., 1991. Injected Xwnt-8 RNA acts early in *Xenopus* embryos to promote formation of a vegetal dorsalizing center. Cell 67, 753–765.

Sokol, S., Christian, J.L., Moon, R.T., Melton, D.A., 1991. Injected Wnt RNA induces a complete body axis in *Xenopus* embryos. Cell 67, 741–752.

Solloway, M.J., Harvey, R.P., 2003. Molecular pathways in myocardial development: a stem cell perspective. Cardiovasc. Res. 58, 264–277.

Solnica-Krezel, L., 2001. Genetic control of convergent extension movements during zebrafish gastrulation. FASEB J. 15, A745.

Tada, M., Smith, J.C., 2000. Xwnt-11 is a target of *Xenopus* Brachyury: regulation of gastrulation movements via dishevelled, but not through the canonical Wnt pathway. Development 127, 2227–2238.

Takada, S., Stark, K.L., Shea, M.J., Vassileva, G., McMahon, J.A., McMahon, A.P., 1994. Wnt-3a regulates somite and tailbud formation in the mouse embryo. Genes Dev. 8, 174–189.

Terami, H., Hidaka, K., Katsumata, T., Iio, A., Morisaki, T., 2004. Wnt11 facilitates embryonic stem cell differentiation to Nkx2.5-positive cardiomyocytes. Biochem. Biophys. Res. Commun. 325, 968–975.

Terry, K., Magan, H., Baranski, M., Burrus, L.W., 2000. Sfrp-1 and sfrp-2 are expressed in overlapping and distinct domains during chick development. Mech. Dev. 97, 177–182.

Tolwinski, N.S., Wieschaus, E., 2004. Rethinking WNT signaling. Trends Genet. 20, 177–181.

Topol, L., Jiang, X., Choi, H., Garrett-Beal, L., Carolan, P.J., Yang, Y., 2003. Wnt-5a inhibits the canonical Wnt pathway by promoting GSK-3-independent beta-catenin degradation. J. Cell Biol. 162, 899–908.

Torres, M.A., Yang-Snyder, J.A., Purcell, S.M., DeMarais, A.A., McGrew, L.L., Moon, R.T., 1996. Activities of the *Wnt-1* class of secreted signaling factors are antagonized by the *Wnt-5A* class and by a dominant negative cadherin in early *Xenopus* embryos. J. Cell Biol. 133, 1123–1137.

Ulrich, F., Krieg, M., Schotz, E.M., Link, V., Castanon, I., Schnabel, V., Taubenberger, A., Mueller, D., Puech, P.H., Heisenberg, C.P., 2005. Wnt11 functions in gastrulation by controlling cell cohesion through Rab5c and E-cadherin. Dev. Cell 9, 555–564.

van den Hoff, M.J.B., Kruithof, B.P.T., Moorman, A.F.M., 2004. Making more heart muscle. BioEssays 26, 248–261.

Veeman, M.T., Axelrod, J.D., Moon, R.T., 2003. A second canon. Functions and mechanisms of beta-catenin-independent Wnt signaling. Dev. Cell 5, 367–377.

Wang, J., Wynshaw-Boris, A., 2004. The canonical Wnt pathway in early mammalian embryogenesis and stem cell maintenance/differentiation. Curr. Opin. Genet. Dev. 14, 533–539.

Wang, S., Krinks, M., Lin, K., Luyten, F.P., Moos-Jr., M., 1997a. Frzb, a secreted protein expressed in the Spemann Organizer, binds and inhibits Wnt-8. Cell 88, 757–766.

Wang, S., Krinks, M., Moos-Jr., M., 1997b. Frzb-1, an antagonist of Wnt-1 and Wnt-8, does not block signaling by Wnts-3a, -5a, or -11. Biochem. Biophys. Res. Commun. 236, 502–504.

Weidinger, G., Moon, R.T., 2003. When Wnts antagonize Wnts. J. Cell Biol. 162, 753–755.

Westfall, T.A., Brimeyer, R., Twedt, J., Gladon, J., Olberding, A., Furutani-Seiki, M., Slusarski, D.C., 2003. Wnt-5/pipetail functions in vertebrate axis formation as a negative regulator of Wnt/beta-catenin activity. J. Cell Biol. 162, 889–898.

Wong, G.T., Gavin, B.J., McMahon, A.P., 1994. Differential transformation of mammary epithelial cells by *Wnt* genes. Mol. Cell. Biol. 14, 6278–6286.

Yamaguchi, T.P., Bradley, A., McMahon, A.P., Jones, S., 1999. A Wnt5a pathway underlies outgrowth of multiple structures in the vertebrate embryo. Development 126, 1211–1223.

Yuasa, S., Itabashi, Y., Koshimizu, U., Tanaka, T., Sugimura, K., Kinoshita, M., Hattori, F., Fukami, S., Shimazaki, T., Okano, H., Ogawa, S., Fukuda, K., 2005. Transient inhibition of BMP signaling by Noggin induces cardiomyocyte differentiation of mouse embryonic stem cells. Nat. Biotechnol. 23, 607–611.

Zakin, L.D., Mazan, S., Maury, M., Martin, N., Guenet, J.L., Brulet, P., 1998. Structure and expression of Wnt13, a novel mouse Wnt2 related gene. Mech. Dev. 73, 107–116.